Development and validation of a method for simultaneous determination of paracetamol, diclofenac and ibuprofen using the Agilent 1120 Compact LC

Application Note

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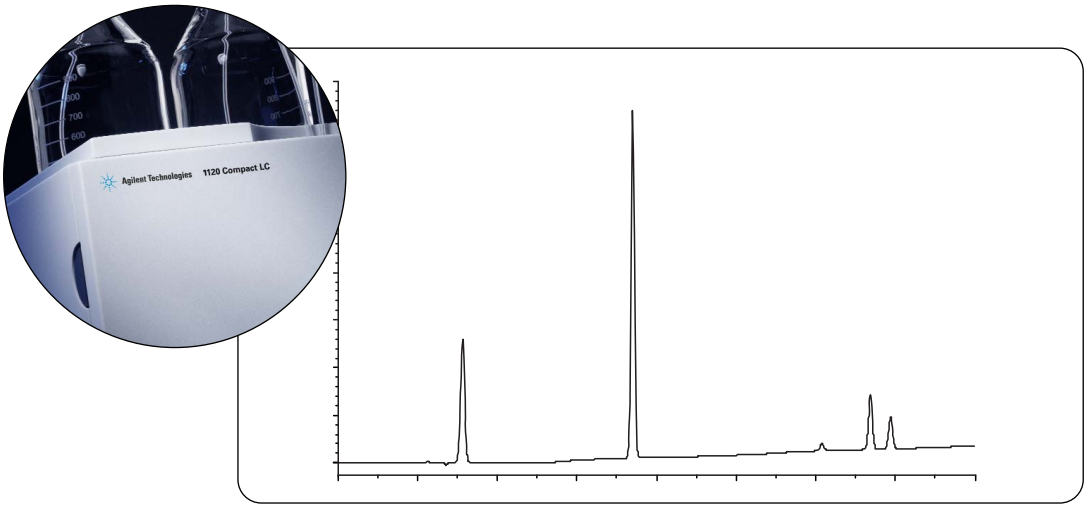

Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. It is an intergrated LC designed for ease of use, performance and reliability. It is ideally suited for the analysis of pharmaceuticals on account of its capability to achieve highly precise retention times and peak areas, and low detection limits for the analyzed compounds. In this Application Note, data is presented that demonstrates:

- Excellent retention time precision < 0.07 % RSD
- Excellent area precision < 1.0 % RSD for baseline separated peaks
- Excellent linearity with coefficient of correlation > 0.9999
- Limit of detection (LOD) 13 – 298 pg for all compounds analyzed

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Introduction

For the routine analysis of pharmaceuticals compounds and impurities in QA/QC it is important to use LC systems that are highly accurate, precise and robust. The Agilent 1120 Compact LC is based on a proven robust design and delivers the required quality of data. This makes it ideally suited for routine QA/QC analysis of pharmaceutical compounds. In this study, the precision, linearity and limits of detection (LOD) of several pharmaceutical compounds were evalutated.

Experimental

Equipment

- Agilent 1120 Compact LC comprising gradient pump with integrated degasser, autosampler with vial tray, column oven and variable wavelength detector, see figure 1
- Agilent TC-C18(2), high carbon load, 150 x 4.6 mm, 5 µm particle size column
- Agilent EZChrom Elite Compact software

Chromatographic conditions

- Mobile phase:
  A: Water + 0.05 % TFA
  B: ACN + 0.045 % TFA
- Gradient:
  0 min, 25 %B;
  1 min, 25 %B;
  4 min, 60 %B;
  7 min, 70 %B
- Flow rate: 1.5 mL/min
- Injection volume: 3 µL
- Column temperature: 40 °C
- Detection wavelength: 230 nm
  Peakwidth: > 0.05 min
- Run time: 8 min
- Post time: 2 min

Results and discussion

Paracetamol, ibuprofen and diclofenac were chosen as example compounds for the evaluation of precision, linearity and LOD. Benzoic acid was used as internal standard (ISTD). Table 1 lists the concentration levels used.
The chromatographic method was set up so that all compounds were baseline separated. Paracetamol posed a particular problem because it elutes from the C-18 phase column with almost no retention. The start conditions were therefore selected so that paracetamol showed at least some retention on the selected column.

Compound	Ibuprofen ng/3 µL	Benzoic acid ng/3 µL	Paracetamol ng/3 µL	Diclofenac ng/3 µL
Level 1	38.15	71.25	56.7	25.2
Level 2	19.075	36.625	28.35	12.6
Level 3	9.538	18.313	14.175	6.3
Level 4	4.769	9.156	7.088	3.15
Level 5	2.384	4.578	3.544	1.575
Level 6	1.192	2.289	1.772	0.788
Level 7	0.596	1.145	0.886	0.394

Table 1
Drug compounds and concentration levels.

Figure 1
Agilent 1120 Compact LC

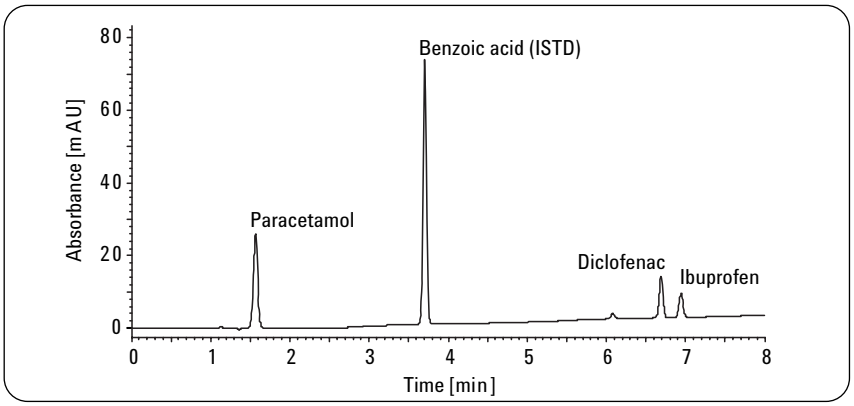

Figure 2
Analysis of pharmaceutical drugs, showing excellent resolution.

Further, the objective of method development was to keep the total analysis time as short as possible. The run and equilibration time could be limited to 10 minutes (figure 2). The mobile phase contained trifluoric acetic acid as modifier. This influenced positively both retention and peak shape. The first gradient slope at 4 minutes was fast enough to keep the time between the first and second peaks as short as possible.

Precision

Analyzing drugs with UV detection means that precision of retention times is of utmost importance. In addition, precision of peak areas must be less than 1 % in the low ng range to be compliant with official regulations. The precision of retention times and areas was determined using level 2 concentration. The results are shown in figure 3 and table 2. In figure 3 ten consecutive runs were overlaid.

Limit of detection

The limit of detection was calculated based on the chromatogram obtained for concentration level 7, see table 3. The limit of detection was in the low 3-digit pg range for ibuprofen and diclofenac, and in the low 2-digit pg range for paracetamol and benzoic acid.

Linearity

Linearity was tested using concentration levels 1 through 7. Figure 4 shows the linearity of paracetamol as an example. The results showed excellent linearity over the entire concentration range. Table 4 shows the correlation coefficients for all compounds.

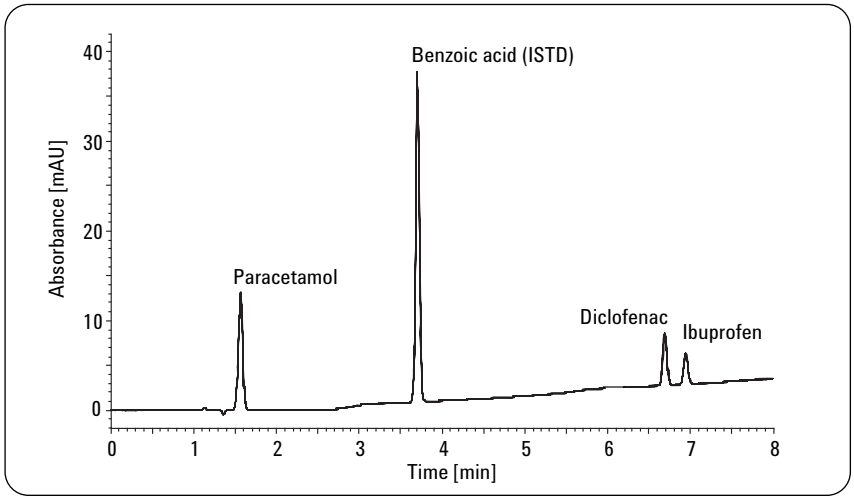

Figure 3
Precision of retention times and areas, showing overlay of 10 consecutive runs.

	% RSD Ret. Times	% RSD Areas
Paracetamol	0.060	0.297
Benzoic acid	0.022	0.179
Diclofenac	0.020	0.807
Ibuprofen	0.016	0.962

Table 2
Precision of retention times and areas for concentration level 2.

	Injected amount (3 µL injection)	Calculated LOD [ng]
Paracetamol	0.394	0.013
Benzoic acid	1.145	0.019
Diclofenac	0.394	0.197
Ibuprofen	0.596	0.298

Table 3
Limits of detection.

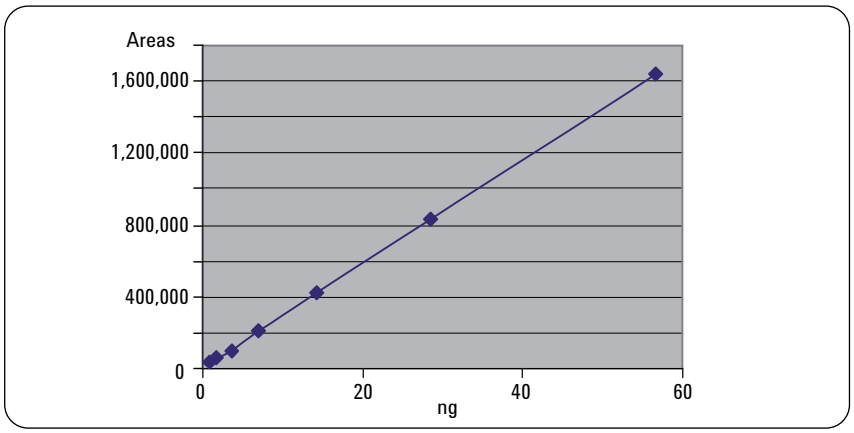

Figure 4
Linearity for paracetamol from 0.886 to 56.7ng per 3 µL injection.

	Correlation coefficient
Paracetamol	0.999966
Benzoic acid	0.999954
Diclofenac	0.999975
Ibuprofen	0.999910

Table 4
Correlation coefficients.

Summary
The Agilent 1120 Compact LC was used for the analysis of pharmaceutical compounds. The instrument was able to analyze these compounds with high precision for retention times and areas. The precision for retention times is less than 0.07 % RSD and less than 1.0 % RSD for areas of baseline separated peaks. The limits of detection were between 13 and 298 pg. The results showed excellent linearity over the tested concentration range and the correlation coefficient was between 0.999910 and 0.999975.

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